

?b 155,357

14mar03 10:06:17 User208669 Session D2229.1

\$0.34 0.096 DialUnits File1

\$0.34 Estimated cost File1

\$0.03 TELNET

\$0.37 Estimated cost this search

\$0.37 Estimated total session cost 0.096 DialUnits

SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1966-2003/Mar W2

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File 357:Derwent Biotech Res. _1982-2003/Mar W2

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*File 357: File is now current. See HELP NEWS 357.

Alert feature enhanced for multiple files, etc. See HELP ALERT.

Set Items Description

? ds

Set Items Description

S1 29757 CMV OR HCMV OR CYTOMEGALOV

S2 1081 DNA (W) VACCINE

S3 122 S1 AND S2

S4 112 RD (unique items)

S5 13396 S1/T1

S6 9 S4 AND S5

S7 115 RHCMV OR (RHESUS AND S1)

S8 113 RD (unique items)

S9 75 US28 OR US(1W)28

S10 26 UL3 OR UL(1W)33

S11 9 UL78 OR UL(1W)78

S12 43 US27 OR US(1W)27

S13 9 S9 AND S10

S14 6 S9 AND S11

S15 9 S9 AND S12

S16 2 S14 NOT S15

S17 0 S13 NOT (S14 OR S15)

S18 64 S9 NOT (S13 OR S14 OR S15)

S19 53 (S10 OR S11 OR S12) NOT S9

?1s6/7/1-6

6/7/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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14550010 22516193 PMID: 12629648

Enhancement of humoral immune responses to a human cytomegalovirus DNA vaccine: Adjuvant effects of aluminum phosphate and CpG oligodeoxynucleotides.

Temperton Nigel J; Quenelle Debra C; Lawson Keirissa M; Zuckerman Jane N; Kern Earl R; Griffiths Paul D; Emery Vincent C

Department of Virology, Royal Free and University College Medical School, London, United Kingdom.

Journal of medical virology (United States) May 2003, 70 (1) p86-90,

ISSN 0146-6615 Journal Code: 7705876

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: In Process

A human cytomegalovirus (HCMV) glycoprotein B (gpUL55) DNA vaccine has been evaluated in BALB/c mice. Intramuscular immunization of these mice with pRc/CMV2-gB resulted in the generation of high levels of gpUL55-specific antibody (geometric mean titer [GMT] 1:8900) and neutralizing antibody (GMT 1:74) after 2 booster doses given 5 and 10 weeks' after primary inoculation. Emulsifying the construct with the aluminum phosphate gel adjuvant Adju-Phos before immunization enhanced gpUL55-specific antibody responses (GMT 1:17800, P = 0.04). Co-immunization with CpG oligodeoxynucleotides was shown to enhance levels of neutralizing antibodies generated by immunization of mice with a pRc/CMV2-gB/Adju-Phos emulsion (P = 0.04). The results provide a rationale for evaluating combinations of other HCMV proteins for incorporation into a multi-target DNA vaccine, and for the optimization of adjuvant usage, to elicit enhanced levels of neutralizing antibodies. J. Med. Virol. 70:86-90, 2003. Copyright 2003 Wiley-Liss, Inc.

Record Date Created: 20030311

6/7/2 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

12942078 21826672 PMID: 11836387

Strong CD8 T-cell responses following coimmunization with plasmids expressing the dominant pp89 and subdominant M84 antigens of murine cytomegalovirus correlate with long-term protection against subsequent viral challenge.

Ye Ming; Morello Christopher S; Spector Deborah H

Molecular Biology Section, Division of Biology, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0366, USA.

Journal of virology (United States) Mar 2002, 76 (5) p2100-12,

ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: T32 AI 07036; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

We previously showed that intradermal immunization with plasmids expressing the murine cytomegalovirus (MCMV) protein IE1-pp89 or M84

protects against viral challenge and that coimmunization has a synergistic protective effect (C. S. Morello, L. D. Cramer, and D. H. Spector, *J. Virol.* 74:3696-3708, 2000). Using an intracellular gamma interferon cytokine staining assay, we have now characterized the CD8+ T-cell response after DNA immunization with pp89, M84, or pp89 plus M84. The pp89- and M84-specific CD8+ T-cell responses peaked rapidly after three immunizations. DNA immunization and MCMV infection generated similar levels of pp89-specific CD8+ T cells. In contrast, a significantly higher level of M84-specific CD8+ T cells was elicited by DNA immunization than by MCMV infection. Fusion of ubiquitin to pp89 enhanced the CD8+ T-cell response only under conditions where vaccination was suboptimal. Three immunizations with either pp89, M84, or pp89 plus M84 DNA also provided significant protection against MCMV infection for at least 6 months, with the best protection produced by coimmunization. A substantial percentage of antigen-specific CD8+ T cells remained detectable, and they responded rapidly to the MCMV challenge. These results underscore the importance of considering antigens that do not appear to be highly immunogenic during infection as DNA vaccine candidates.

Record Date Created: 20020211

6/7/3 (Item 3 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

10825828 20349085 PMID: 10892996

Immunogenicity evaluation of DNA vaccines that target guinea pig cytomegalovirus proteins glycoprotein B and UL83.

Schleiss M R; Bourne N; Jensen N J; Bravo F; Bernstein D I

Division of Infectious Diseases, Children's Hospital Research Foundation,

Cincinnati, Ohio 45229, USA. schlm0@chmcc.org

Viral immunology (UNITED STATES) 2000, 13 (2) p155-67, ISSN

0882-8245 Journal Code: 8801552

Contract/Grant No.: AI 01276-01; AI; NIAID; AI 65289; AI; NIAID; ROI

HD38416-01; HD; NICHD

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Vaccines are needed for control of congenital human cytomegalovirus (HCMV) infection. Although the species-specificity of cytomegaloviruses precludes preclinical evaluation of HCMV vaccines in animal models, the guinea pig cytomegalovirus (GPCMV), which causes disease in utero, is a relevant model for the study of vaccines against congenital infection. We investigated whether DNA vaccines that target two GPCMV proteins, glycoprotein B (gB) and UL83 (pp65), are capable of eliciting immune responses in vivo. After cloning each gene into an expression vector, DNA was delivered by intramuscular inoculation and by pneumatic epidermal delivery. In Swiss-Webster mice, anti-gB titers were significantly higher

after epidermal delivery. After epidermal inoculation in guinea pigs, all gB-immunized animals (n = 6) had antibody responses comparable to those induced by natural infection. Viral neutralization titers ranged from 1:64 to greater than 1:128. A GPCMV UL83 DNA vaccine also elicited an antibody response in all immunized guinea pigs (n = 6) after epidermal administration. Immunoprecipitation and Western blot assays confirmed that immune sera were immunoreactive with virion-associated UL83 and gB proteins. We conclude that DNA vaccines against GPCMV structural proteins are immunogenic, and warrant further investigation in the guinea pig model of congenital CMV infection.

Record Date Created: 20001107

6/7/4 (Item 4 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

10663016 20193809 PMID: 10729145

Suppression of murine cytomegalovirus (MCMV) replication with a DNA vaccine encoding MCMV M84 (a homolog of human cytomegalovirus pp65).

Morello C S; Cramer L D; Spector D H

Department of Pathology, University of California, San Diego, La Jolla, California 92093-0366, USA.

Journal of virology (UNITED STATES) Apr 2000, 74 (8) p3696-708,

ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: AI20954; AI; NIAID; GM07198; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The cytotoxic T-lymphocyte (CTL) response against the murine cytomegalovirus (MCMV) immediate-early gene 1 (IE1) 89-kDa phosphoprotein pp89 plays a major role in protecting BALB/c mice against the lethal effects of the viral infection. CTL populations specific to MCMV early-phase and structural antigens are also generated during infection, but the identities of these antigens and their relative contributions to overall immunity against MCMV are not known. We previously demonstrated that DNA vaccination with a pp89-expressing plasmid effectively generated a CTL response and conferred protection against infection (J. C. Gonzalez Armas, C. S. Morello, L. D. Cramer, and D. H. Spector, *J. Virol.* 70:7921-7928, 1996). In this report, we have sought (i) to identify other viral antigens that contribute to immunity against MCMV and (ii) to determine whether the protective response is haplotype specific. DNA immunization was used to test the protective efficacies of plasmids encoding MCMV homologs of human cytomegalovirus (HCMV) tegument (M32, M48, M56, M82, M83, M69, and M99), capsid (M85 and M86), and nonstructural antigens (IE1-pp89 and M84). BALB/c (H-2(d)) and C3H/HeN (H-2(k)) mice were immunized by intradermal injection of either single plasmids or cocktails of up to four expression plasmids and then challenged with sublethal doses

of virulent MCMV administered intraperitoneally. In this way, we identified a new viral gene product, M84, that conferred protection against viral replication in the spleens of BALB/c mice. M84 is expressed early in the infection and encodes a nonstructural protein that shares significant amino acid homology with the HCMV UL83-pp65 tegument protein, a major target of protective CTLs in humans. Specificity of the immune response to the M84 protein was confirmed by showing that immunization with pp89 DNA, but not M84 DNA, protected mice against subsequent infection with an MCMV deletion mutant lacking the M84 gene. The other MCMV genes tested did not generate a protective response even when mice were immunized with vaccinia viruses expressing the viral proteins. However, the M84 plasmid was protective when injected in combination with nonprotective plasmids, and coimmunization of BALB/c mice with pp89 and M84 provided a synergistic level of protection in the spleen. Viral titers in the salivary glands were also reduced, but not to the same extent as observed in the spleen, and the decrease was seen only when the BALB/c mice were immunized with pp89 plus M84 or with pp89 alone. The experiments with the C3H/HeN mice showed that the immunity conferred by DNA vaccination was haplotype dependent. In this strain of mice, only pp89 elicited a protective response as measured by a reduction in spleen titer. These results suggest that DNA immunization with the appropriate combination of CMV genes may provide a strategy for improving vaccine efficacy.

Record Date Created: 20000426

6/7/5 (Item 5 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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10298514 99268525 PMID: 10338203

Induction of neutralizing antibody against human cytomegalovirus (HCMV) with DNA-mediated immunization of HCMV glycoprotein B in mice.

Hwang E S; Kwon K B; Park J W; Kim D J; Park C G; Cha C Y

Department of Microbiology, Seoul National University College of Medicine, Republic of Korea.

Microbiology and immunology (JAPAN) 1999, 43 (3) p307-10, ISSN 0385-5600 Journal Code: 7703966

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Immunization was accomplished by inoculating pcGB containing human cytomegalovirus (HCMV) glycoprotein B (gB) gene into BALB/c mice intramuscularly. IgM antibody was detected in all the immunized group. IgG antibody was also found in all the tested mice with a mean peak antibody titer of 1:262 in three-times immunized groups. IgG antibody appeared at 2 weeks postinoculation, raised peak levels at 7 weeks postinoculation and persisted over 6 months. Neutralizing antibody was developed, and the percent reduction of input infectivity in 1:100 diluted sera was 74.5 % in

three-times immunized groups. This study suggested that DNA vaccine using the gene encoding HCMV gB is a candidate method for developing immunity to HCMV.

Record Date Created: 19990903

6/7/6 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

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0301702 DBR Accession No.: 2003-03487 PATENT

Novel mutant cytomegalovirus pp65 protein which lacks protein kinase activity and elicits cytotoxic T lymphocyte response against cells infected with the virus, useful for enhancing immunity to the virus - vector-mediated gene transfer and expression in host cell for recombinant vaccine, nucleic acid vaccine and gene therapy

AUTHOR: ZAIA J A; HAWKINS G

PATENT ASSIGNEE: ZAIA J A; HAWKINS G 2002

PATENT NUMBER: US 20020081318 PATENT DATE: 20020627 WPI ACCESSION NO.:

2002-690114 (200274)

PRIORITY APPLIC. NO.: US 815330 APPLIC. DATE: 20010323 NATIONAL APPLIC. NO.: US 815330 APPLIC. DATE: 20010323

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A mutant cytomegalovirus (CMV) pp65

protein (I) which lacks protein kinase activity, where (I) elicits a cytotoxic T lymphocyte (CTL) response against cells infected with CMV, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) DNA (II) encoding (I); (2) DNA (III) which comprises pcDNAmp65mII; (3) vaccine composition (IV) which comprises (I) and a pharmaceutically acceptable carrier; (4) cellular vaccine composition (V) which comprises antigen presenting cells (APC) that have been treated in vitro so as to present epitopes of (I) and a pharmaceutically acceptable carrier; (5) DNA vaccine composition (VI) which comprises (II) or (III) and a pharmaceutically acceptable carrier; (6) eukaryotic virus vector (VII) which comprises (II) or (III); (7) recombinant live virus vaccine (VIII) which comprises (II) or (III); and (8) diagnostic agent (IX) for detecting the presence of active versus quiescent CMV infections which comprises pp65mII transfect target APC. BIOTECHNOLOGY - Preferred Protein: (I) contains the K436N mutation. Preferred Vaccine: The DNA vaccine composition comprising (III), further comprises an adjuvant. Preparation: (I) is prepared by standard recombinant cloning techniques followed by expression in bacterial cells. ACTIVITY - Virucide; Immunostimulant. No supporting data provided. MECHANISM OF ACTION - Vaccine; Gene therapy. No supporting data is given. USE - (III), (IV), (V), (VI) or (VIII) are useful for enhancing immunity against CMV or for vaccinating against CMV (claimed). ADMINISTRATION - No administration or dosage information

given. **EXAMPLE** - DNA constructions and generation of recombinant vaccinia virus was as follows. The cytomegalovirus (CMV)pp65 gene, cloned into the BamHI and EcoRI restriction sites of pBluescript II KS DNA was used to create the constructs. CMV nucleotide coordinates were used. Mutations of the CMVpp65 DNA were made using the following pairs of mutagenic primers: 5'-GGGGGGCCGCAACCGCAAAATCAGCATCC-3' and 5'-GGATGCTGATTTGGCGGTGCGGGCCCGC-3', and 5'-GAGTCCACCGTCGGCCCAAGAGAGACAC

CGACGAG-3' and 5'-CTCGTGGTGTCTCTTTGGGGGCGACGGTGGACTC-3'. The mutations were confirmed by DNA sequencing. A negative control lacking the putative CMVpp65 phosphokinase domain was created in pBluescript II KS (pp65mTTH) by digesting the CMVpp65 gene with Tth111 and NsiI to delete the nucleotides which encode CMVpp65. Overhanging sequences were removed with mung bean nuclease and the ends were re-ligated using T4 DNA ligase. To express the proteins in a bacterial system, the CMVpp65 DNA was removed from pBluescript using Sal I-Bam HI digestion and inserted downstream from the CMV promoter in the pQE9 vector. The pQE9 CMVpp65 expression plasmid was used to transform *Escherichia coli* strain M15, which contained the repressor pREP4 plasmid, and the proteins were expressed. The mTTH modification of CMVpp65 was created to establish a kinase-deficient CMVpp65 control by inserting the Tth111 and NsiI truncations into the pQE9 plasmid. The plasmid containing the native CMVpp65 was designated pQE9pp65n, the K436N subdomain II mutant was pQE9pp65mII, the combined K436N/E465K mutant was pQE9pp65mVIII, and the truncation control was pQE9pp65mTTH. pQE9 pp150 was used as a negative plasmid control. The CMVpp65 mutant sequences were subcloned into the transfer vector pSC11 and then transfected into CV-1 cells that had been simultaneously infected with wild-type WR strain vaccinia virus. Recombinant virus was cloned and correct insertion was confirmed by polymerase chain reaction (PCR) and DNA sequencing. The efficiency of protein expression in the constructs was verified by western blot. The proteins were purified from cells transfected with the indicated pQE9 vector, separated using 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a nitrocellulose membrane and probed with monoclonal antibody (mAb) 28-103 specific for the detection of the pp65 protein. All the proteins of the constructs bearing the intact carboxy-terminus of CMVpp65 were detected by the mAb, including the mutant CMVpp65. The level of expression was qualitatively similar in all constructs, suggesting that the mutations did not alter protein expression. (21 pages)

8/7/25 27 31 37 42 43 48 96 99 108

8/7/25 (Item 25 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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10641947 20171829 PMID: 10704336

Primate cytomegaloviruses encode and express an IL-10-like protein.

Lockridge K M; Zhou S S; Kravitz R H; Johnson J L; Sawai E T; Blewett E L; Barry P A

Center for Comparative Medicine, University of California-Davis, Davis, California, 95616, USA. kmloefer@ucdavis.edu

Virology (UNITED STATES) Mar 15 2000, 268 (2) p272-80, ISSN 0042-6822 Journal Code: 0110674

Contract/Grant No.: P51 RR-AG00169; RR; NCRR; RO1 HD-57883; HD; NICHD; RO1 NS-36859; NS; NINDS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

An open reading frame (ORF) with homology to interleukin-10 (IL-10) has been identified in rhesus cytomegalovirus (RhCMV). The IL-10-like protein is generated from a multisplliced, polyadenylated early gene transcript encompassing part of the corresponding UL111A ORF of human CMV (HCMV). Immunological analyses confirm expression of the IL-10-like protein both in tissue culture and in RhCMV-infected rhesus macaques. Conserved ORFs were subsequently identified in human, baboon, and African green monkey CMV, and a fully processed transcript has been mapped in fibroblasts infected with the Towne strain of HCMV. The conservation of this previously unrecognized ORF suggests that the protein may play an essential role in primate CMV persistence and pathogenesis. Copyright 2000 Academic Press.

Record Date Created: 20000418

8/7/27 (Item 27 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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10458573 99445865 PMID: 10516066

Pathogenesis of experimental rhesus cytomegalovirus infection.

Lockridge K M; Sequar G; Zhou S S; Yue Y; Mandell C P; Barry P A

Center for Comparative Medicine, Department of Medical Pathology, University of California-Davis, Davis, California.

Journal of virology (UNITED STATES) Nov 1999, 73 (11) p9576-83, ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: P51 RR-AG00169; RR; NCRR; RO1 HL-57883; HL; NHLBI; RO1 NS-36859; NS; NINDS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human cytomegalovirus (HCMV) establishes and maintains a lifelong persistence following infection in an immunocompetent host. The determinants of a stable virus-host relationship are poorly defined. A nonhuman primate model for HCMV was used to investigate virological and host parameters of infection in a healthy host. Juvenile rhesus macaques

(*Macaca mulatta*) were inoculated with rhesus cytomegalovirus (RhCMV), either orally or intravenously (i.v.), and longitudinally necropsied. None of the animals displayed clinical signs of disease, although hematologic abnormalities were observed intermittently in i.v. inoculated animals. RhCMV DNA was detected transiently in the plasma of all animals at 1 to 2 weeks postinfection (wpi) and in multiple tissues beginning at 2 to 4 wpi. Splenic tissue was the only organ positive for RhCMV DNA in all animals. The location of splenic cells expressing RhCMV immediate-early protein 1 (IE1) in i.v. inoculated animals changed following inoculation. At 4 to 5 wpi, most IE1-positive cells were perifollicular, and at 25 wpi, the majority were located within the red pulp. All animals developed anti-RhCMV immunoglobulin M (IgM) antibodies within 1 to 2 wpi and IgG antibodies within 2 to 4 wpi against a limited number of viral proteins. Host reactivity to RhCMV proteins increased in titer (total and neutralizing) and avidity with time. These results demonstrate that while antiviral immune responses were able to protect from disease, they were insufficient to eliminate reservoirs of persistent viral gene expression.

Record Date Created: 19991104

8/7/31 (Item 31 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

10147688 99118729 PMID: 9921756

Immunohistochemical studies of productive rhesus cytomegalovirus infection in rhesus monkeys (*Macaca mulatta*) infected with simian immunodeficiency virus.

Kuhn E M; Stolle N; Matz-Rensing K; Mach M; Stahl-Henning C; Hunsmann G; Kaup F J

German Primate Centre, Göttingen. evakuhn@bprc.nl

Veterinary pathology (UNITED STATES) Jan 1999, 36 (1) p51-6, ISSN 0300-9858 Journal Code: 0312020

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In humans infected with the human immunodeficiency virus (HIV), clinical disease due to human cytomegalovirus (HCMV) infection is one of the AIDS-defining diseases; HCMV is the most common opportunistic infection found postmortem. Histologically, the typical lesions are characterized by "owl's eye cells." In rhesus monkeys infected with simian immunodeficiency virus (SIV), comparable lesions are caused by an infection with the rhesus CMV (RhCMV). The aim of this study was to investigate the incidence of productive and latent RhCMV infection in monkeys infected with SIV macaques (SIVmac). Eleven SIVmac-infected rhesus monkeys, which were euthanized after developing AIDS-like disease, and 11 clinically healthy and uninfected animals comprised the study. The monkeys were screened serologically for RhCMV by western-blot analysis. Immunohistochemistry was

performed by an indirect immunoperoxidase technique with a polyclonal rabbit RhCMV-antiserum. Lesions characteristic of RhCMV-associated diseases were detected histologically. All animals were latently RhCMV-infected. Seven of eleven (63.6%) SIV-infected macaques were productively RhCMV infected according to immunohistochemistry. RhCMV antigen was identified in the gastrointestinal tract, the hepatobiliary system, the lungs, and the testicles. Two of these seven animals showed characteristic inflammatory lesions associated with productive infection. Consequently, the CMV prevalence in SIVmac-infected rhesus monkeys and human AIDS patients is comparable.

Record Date Created: 19990413

8/7/37 (Item 37 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09687267 98118459 PMID: 9454707

Characterization of rhesus cytomegalovirus genes associated with anti-viral susceptibility.

Swanson R; Berguam E; Wong S W

Division of Pathobiology and Immunology, Oregon Regional Primate Research Center, Beaverton 97006, USA.

Virology (UNITED STATES) Jan 20 1998, 240 (2) p338-48, ISSN 0042-6822 Journal Code: 0110674

Contract/Grant No.: RR00163; RR; NCRR

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Studies were initiated to determine whether rhesus cytomegalovirus (RhCMV)-infected macaques could serve as an animal model for evaluating anti-CMV compounds, as macaques have a naturally occurring CMV that is similar to human CMV (HCMV). Utilizing plaque reduction assays, RhCMV was tested to anti-viral susceptibility. By these assays, RhCMV displayed anti-viral susceptibility to ganciclovir at a 50% effective dose (ED50) of 0.8 micromM, acyclovir at an ED50 of 15 micromM, and foscarnet at an ED50 of 250 micromM. By Southern blot analysis with HCMV-UL97 (phosphotransferase) and DNA polymerase (pol) genes as probes, we isolated viral DNA fragments that strongly hybridized. DNA sequence analysis of these DNA fragments revealed two open reading frames with homology to HCMV UL97 and DNA polymerase. Steady-state RNA analysis revealed that the RhCMV UL97 homologue and pol genes are transcribed as early late and early genes, respectively. Comparison against HCMV showed the RhCMV UL97 homologue exhibits 54.4% amino acid (aa) sequence identity to HCMV UL97 and the RhCMV DNA polymerase 59.2% aa sequence identity to HCMV DNA polymerase. Results from anti-viral assays and molecular characterization of these two viral genes suggest that RhCMV-infected rhesus macaques should serve as an excellent animal model for evaluating future anti-CMV compounds.

Record Date Created: 19980220

8/7/42 (Item 42 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09501147 97410286 PMID: 9267001

Cloning and characterization of rhesus cytomegalovirus glycoprotein B.

Kravitz R H; Scibica K S; Cho K; Luciw P A; Barry P A

Department of Medical Pathology, University of California, Davis 95616, USA.

Journal of general virology (ENGLAND) Aug 1997, 78 (Pt 8) p2009-13, ISSN 0022-1317 Journal Code: 0077340

Contract/Grant No.: RR00169, RR, NCR

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Rhesus cytomegalovirus (RhCMV) infection of rhesus macaques is an important model to investigate critical issues of cytomegalovirus biology. To better understand host immunological responses to viral glycoproteins, the glycoprotein B (gB) gene of RhCMV was molecularly cloned, sequenced and characterized. Transcription analysis revealed that RhCMV gB was transcribed as a late gene. The RhCMV gB gene encoded a predicted protein of 854 amino acids that was 60% identical/75% similar to the human CMV (HCMV) gB protein. The region of HCMV gB proposed to be responsible for virus binding to host cells, fusion and cell-to-cell spread was the most highly conserved region with RhCMV gB (74% identity/85% similarity). Conserved elements included 11 of 12 cysteine residues, 14 of 16 potential N-linked glycosylation sites and cross-reactive epitopes. Metabolic labelling experiments demonstrated that RhCMV gB was proteolytically processed similarly to HCMV gB. These results are critical for investigating virus-host relationships in CMV-infected primates.

Record Date Created: 19970908

8/7/43 (Item 43 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09501146 97410285 PMID: 9267000

Identification of the gene coding for rhesus cytomegalovirus glycoprotein B and immunological analysis of the protein.

Kropff B; Mach M

Institut für Klinische und Molekulare Virologie, Universität Erlangen-Nürnberg, Erlangen, Germany.

Journal of general virology (ENGLAND) Aug 1997, 78 (Pt 8) p1999-2007, ISSN 0022-1317 Journal Code: 0077340

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The nucleotide sequence of the gene encoding glycoprotein B (gB) of rhesus cytomegalovirus (RhCMV) was determined and the protein characterized. The open reading frame of gB encoded a protein of 854 amino acids with 60% identity and 75% similarity at the amino acid level to human cytomegalovirus (HCMV) gB. Cysteine residues in the extraluminal part of the protein are perfectly conserved. Out of the 16 potential N-linked glycosylation sites present in HCMV gB, 15 are conserved in RhCMV gB. Immunoblot analyses with antisera detected three bands of 150 kDa, 90-110 kDa and 55 kDa representing the full-length gB as well as the proteolytic cleavage products. Cross-reactivity and cross-neutralization of a number of HCMV gB-specific monoclonal antibodies with RhCMV gB indicated sharing of immunogenic epitopes between the two molecules. The RhCMV gB regions corresponding to antigenic domains AD-1, 2 and 3 of HCMV gB were immunogenic during natural RhCMV infection with the AD-1 region being the immunodominant domain. The data indicate that RhCMV might represent a useful model to investigate pathogenesis and immune surveillance of cytomegaloviruses.

Record Date Created: 19970908

8/7/48 (Item 48 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09141578 97048052 PMID: 8892893

Cytotoxic T-lymphocyte responses to cytomegalovirus in normal and simian immunodeficiency virus-infected rhesus macaques.

Kaur A; Daniel M D; Hempel D; Lee-Parritz D; Hirsch M S; Johnson R P

Division of Immunology, New England Regional Primate Center, Southborough, Massachusetts 01772, USA.

Journal of virology (UNITED STATES) Nov 1996, 70 (11) p7725-33, ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: D43TW00004; TW; FIC; RR 00055; RR; NCR; RR 00168; RR ; NCR

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Disseminated cytomegalovirus (CMV) infection is a frequent occurrence in human immunodeficiency virus-infected humans and in simian immunodeficiency virus (SIV)-infected rhesus macaques. Rhesus macaques are a suitable animal model with which to study in vivo interactions between CMV and AIDS-associated retroviruses. Since cytotoxic T lymphocytes (CTL) play a major role in control of viral infections, we have characterized CMV-specific CTL responses in SIV-infected and uninfected rhesus macaques. Autologous fibroblasts infected with rhesus CMV were used to stimulate freshly isolated peripheral blood mononuclear cells from CMV-seropositive

animals. Following in vitro stimulation, specific CTL activity against CMV-infected autologous fibroblasts was detected in CMV-seropositive but not in CMV-seronegative normal macaques. CMV-specific CTL activity comparable to that in normal animals was also detected in two CMV-seropositive macaques infected with a live attenuated SIV strain (SIVdelta3) and in two of three macaques infected with pathogenic SIV strains. The CMV-specific CTL response was class I major histocompatibility complex restricted and mediated by CD8+ cells. An early CMV protein(s) was the dominant target recognized by bulk CTL, although the pattern of CTL recognition of CMV proteins varied among animals. Analysis of CMV-specific CTL responses in macaques should serve as a valuable model for CMV immunopathogenesis and will facilitate prospective in vivo studies of immune interactions between CMV and SIV in AIDS.

Record Date Created: 19961230

8/7/96 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

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0300289 DBR Accession No.: 2003-02073 PATENT

Recombinant cytomegalovirus with a CMV genome with a first heterologous nucleotide sequence encoding a heterologous chemokine element, useful for inducing an immune response, and in therapeutic or prophylactic treatments - vector-mediated gene transfer and expression in host cell for nucleic acid vaccine and gene therapy
 AUTHOR: SCHALL T J; PENFOLD M E T
 PATENT ASSIGNEE: CHEMOCENTRYX INC 2002
 PATENT NUMBER: WO 200262296 PATENT DATE: 20020815 WPI ACCESSION NO.:
 2002-643385 (200269)

PRIORITY APPLIC. NO.: US 265925 APPLIC. DATE: 20010202

NATIONAL APPLIC. NO.: WO 2002US3228 APPLIC. DATE: 20020201

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A new recombinant cytomegalovirus

(CMV) (1) comprising a CMV genome with a first heterologous nucleotide sequence encoding a heterologous chemokine element, and a second heterologous nucleotide sequence encoding an immunogenic polypeptide, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a recombinant CMV comprising a genome with a heterologous nucleotide sequence encoding a heterologous chemokine or ligand, or an immunogenic polypeptide; (2) inducing (M1) an immune response in a host comprising administering a composition of (1); (3) therapeutic or prophylactic treatment (M2) comprising administering a composition of (1) to an animal, where the immunogenic polypeptide comprises an antigen correlated with a disease or infection which the animal has or is susceptible to obtaining, and where the administration induces an immune response in the animal; and (4) preparing (M3) (1).

BIOTECHNOLOGY - Preferred Cytomegalovirus: The CMV genome in (1) is encapsulated in infectious form as a virion, and attenuated to reduce virulence in a host. The viral dissemination gene is disabled, and is a gene encoding a viral chemokine element or a viral immune-modulatory gene. The gene encoding the viral chemokine element is selected from US28, US27, UL33, UL78, UL146, UL147, MCK-1 and MCK-2, or their homolog. The viral immune-modulatory gene is selected from UL111A, US3, US6, US11, US2, UL83, UL18, UL40, m144, m152, m04, m06 and m138, or their homolog. The host is selected from a non-human primate or commercial livestock. The mammal is a rhesus monkey or a mouse. The heterologous chemokine element is endogenous to the host, and is a chemokine ligand or receptor. The CMV genome is attenuated to reduce virulence in a mammal and the heterologous chemokine element is selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog. The immunogenic polypeptide comprises an antigen from a pathogenic organism or a tumor antigen. The pathogenic organism is a bacterium, a virus or a parasite. The immunogenic polypeptide further comprises a fragment of a polypeptide from organisms selected from *Bacillus anthracis*, Dengue, *Yersinia pestis*, Ebola, Marburg, Lassa, Venezuelan Equine Encephalitis or Eastern Equine Encephalitis. The immunogenic polypeptide preferably comprises a tumor antigen, and is selected from antigens associated with breast cancers, lung cancers, thyroid carcinomas, squamous cell carcinomas or renal cell carcinomas. The first and second heterologous nucleotide sequence are operably linked to a or different promoters that is operative in the host. The construct is formulated as a composition, the composition comprising the construct and an adjuvant, carrier, diluent or excipient. (1) further comprises: (a) the immunogenic polypeptide comprising an antigen from an organism that is pathogenic in humans or a human tumor antigen; (b) a viral chemokine element or a viral immune-modulatory gene is disabled, the viral chemokine element selected from US28, US27, UL33, UL78, UL146 and UL147 and the viral immune-modulatory gene selected from UL111A, US3, US6, US11, US2, UL83, UL18 and UL40; and (c) the heterologous chemokine element is selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog. The CMV genome in the recombinant virus is attenuated by virtue of a disabled viral dissemination gene and/or a viral immune-modulatory gene, and is attenuated to reduce virulence in a host and encapsulated in infectious form. The immunogenic polypeptide comprises an antigen from an organism that is pathogenic in a host or a tumor antigen. Preferred Method: The CMV genome in (M1) is attenuated to reduce virulence in the host, and is disabled. The viral dissemination gene is a gene encoding a viral chemokine element or a viral immune-modulatory gene. The gene encoding the viral chemokine element is selected from US28, US27, UL33, UL78, UL146, UL147, MCK-1 and MCK-2, or their homolog. The viral immune-modulatory gene is selected from UL111A, US3, US6, US11, US2, UL83, UL18, UL40, m144, m152, m04, m06 and m138, or a homolog. The

heterologous chemokine element is endogenous to the host. The heterologous chemokine element is a chemokine ligand or receptor. The host is a mammal and the heterologous chemokine element is selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog. The pathogenic organism is a bacterium, a virus or a parasite. (M1) further comprises: (a) the host is a human; (b) a viral chemokine element or a viral immune-modulatory gene is disabled, the viral chemokine element selected from US28, US27, UL33, UL78, UL146, and UL147 and the viral immune-modulatory gene selected from UL111A, US3, US6, US11, US2, UL83, UL18 and UL40; (c) the heterologous chemokine element is selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog; and (d) the immunogenic polypeptide comprises an antigen from an organism that is pathogenic in humans or a human tumor antigen. The method alternatively comprises: (a) the host is a rhesus monkey; (b) a viral chemokine element or a viral immune-modulatory gene is disabled, the viral chemokine element selected from rhUS28.1, rhUS28.2, rhUS28.3, rhUS28.4, rhUS28.5, rhUL33 and rhUL78 and the viral immune-modulatory gene selected from rhUL111A, US3, US6, US11, US2, UL83 and UL40; (c) the heterologous chemokine element is selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog; and (d) the immunogenic polypeptide comprises an antigen from a pathogenic organism or a tumor antigen. The method further comprises: (a) the host is a mouse; (b) a viral chemokine element or a viral immune-modulatory gene is disabled, the viral chemokine element selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog; and (d) the immunogenic polypeptide comprises an antigen from a pathogenic organism or a tumor antigen. The method further comprises: (a) the host is a mouse; (b) a viral chemokine element or a viral immune-modulatory gene is disabled, the viral chemokine element selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog; (c) the heterologous chemokine element is selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog; (d) the immunogenic polypeptide comprises an antigen from a pathogenic organism or a tumor antigen. The CMV genome in (M2) is attenuated by virtue of a disabled viral dissemination gene. The viral dissemination gene is a gene encoding a viral chemokine element or a viral immune-modulatory gene. The gene encoding the viral chemokine element is selected from US28, US27, UL33, UL78, UL146, UL147, MCK-1 and MCK-2, or their homolog. The viral immune-modulatory gene is selected from UL111A, US3, US6, US11, US2, UL83, UL18, UL40, m144, m152, m04, m06 and m138, or a homolog. The animal is selected from a human, a non-human primate and commercial livestock. The mammal is from a non-human primate and a mouse. The method further comprises: (a) the host is a human; (b) a viral chemokine element or a viral immune-modulatory gene is disabled, the viral chemokine element selected from US28, US27, UL33, UL78, UL146, and UL147 and the viral immune-modulatory gene selected from UL111A, US3, US6, US11, US2, UL83, UL18 and UL40; (c) the heterologous chemokine element is selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog; and (d) the immunogenic polypeptide comprises an antigen from an organism that is pathogenic in humans or a human tumor antigen. The method alternatively comprises:

(a) the host is a rhesus monkey; (b) a viral chemokine element or a viral immune-modulatory gene is disabled, the viral chemokine element selected from rhUS28.1, rhUS28.2, rhUS28.3, rhUS28.4, rhUS28.5, rhUL33 and rhUL78 and the viral immune-modulatory gene selected from rhUL111A, US3, US6, US11, US2, UL83 and UL40; (c) the heterologous chemokine element is selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog; and (d) the immunogenic polypeptide comprises an antigen from a pathogenic organism or a tumor antigen. The method further comprises: (a) the host is a mouse; (b) a viral chemokine element or a viral immune-modulatory gene is disabled, the viral chemokine element selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog; and (d) the immunogenic polypeptide comprises an antigen from a pathogenic organism or a tumor antigen. (M3) further comprises attenuating the CMV genome to reduce virulence in a host. The attenuation comprises disabling a viral dissemination gene. The viral dissemination gene is a gene encoding a viral chemokine element or a viral immune-modulatory gene. The gene encoding the viral chemokine element is selected from US28, US27, UL33, UL78, UL146, UL147, MCK-1 and MCK-2, or their homolog. The viral immune-modulatory gene is selected from UL111A, US3, US6, US11, US2, UL83, UL18, UL40, m144, m152, m04, m06 and m138, or their homolog. The host is selected from a human, a non-human primate and commercial livestock. The mammal is selected from the group consisting of a rhesus monkey and a mouse. The heterologous chemokine element is endogenous to the host, and is a chemokine ligand or receptor. The heterologous chemokine element is selected from MIP3alpha, SLC, MDC, MC10, MIP1beta, ELC and CCR7, or their homolog. The immunogenic polypeptide comprises an antigen from a pathogenic organism or a tumor antigen. The pathogenic organism is a bacterium, a virus or a parasite. The method further comprises inserting at least one promoter that is operative in the host into the CMV genome such that the at least one promoter is operably linked to the first and second heterologous nucleotide sequence. The method alternatively comprises inserting at least two promoters that are operative in the host into the CMV genome such that the first and second heterologous nucleotide sequence are operably linked to different promoters. The method further comprises combining (i) with a carrier, diluent or excipient. ACTIVITY - Virucide. No suitable data given. MECHANISM OF ACTION - Gene therapy; Vaccine. USE - (i) are useful for inducing an immune response in a host and in treatment methods, either therapeutically or prophylactically (claimed). ADMINISTRATION - The vaccine preparations dose are from 10 to 107 pfu per dose, preferably 103-106. Routes of administration include intradermal, subcutaneous, oral, intramuscular, intraperitoneal and transdermal. EXAMPLE - Experimental protocols are described but no results are given. (74 pages)

8/7/99 (Item 4 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

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0291312 DBR Accession No.: 2002-13159 PATENT

Isolated or recombinant homologs of US28 proteins and nucleic acids

encoding the proteins, for use in vaccine compositions for treating an animal infected with, or at risk of infection by, cytomegalovirus - vector expression in host cell for recombinant protein gene production, antibody, transgenic animal, sense, antisense oligonucleotide, ribozyme useful in virus infection gene therapy and vaccine

AUTHOR: SCHALL T J; PENFOLD M

PATENT ASSIGNEE: CHEMOCENTRYX INC 2002

PATENT NUMBER: WO 200218954 PATENT DATE: 20020307 WPI ACCESSION

NO.:

2002-351718 (200238)

PRIORITY APPLIC. NO.: US 229365 APPLIC. DATE: 20000830

NATIONAL APPLIC. NO.: WO 2001US27392 APPLIC. DATE: 20010830

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated or recombinant homolog of

US28 protein (I) which binds a chemokine (encoded by an open reading frame in the unique short (US) region in human cytomegalovirus (CMV) genome), having at least 75% identity to a sequence (S1) fully defined in the specification, over a region of at least 40 amino acids in length, or a protein comprising at least 12 amino acids of S1, is new.

DETAILED DESCRIPTION - S1 comprises a sequence of 344, 339, 340, 328, 485, 401, 365 or 423 amino acids fully defined in the specification

(amino acid sequences of rhesus monkey proteins RhUS28.1, RhUS28.2,

RhUS28.3, RhUS28.4, RhUS28.5, RhUL78 (encoded by an open reading frame

78 in the unique long (UL) region of CMV genome), RhUL33 and RhUL33

spliced, respectively). INDEPENDENT CLAIMS are also included for the

following: (1) an isolated, purified or recombinant nucleic acid (II)

encoding (I) (US28 homolog); (2) a vector comprising (II); (3) a cell

comprising (II); (4) a vaccine (III) comprising an immunogenic CMV

polypeptide encoded by at least a region of CMV genome in which the

polynucleotide sequence encoding US28 or its homolog has been

inactivated; (5) identifying (M1) an agent that reduces CMV

dissemination in an animal, by determining whether the agent (A)

inhibits the expression or activity of US28 or US28 homolog, or

fragment or variant of US28 or US28 homolog; and (6) treating (M2) an

animal infected with CMV or at risk of infection by CMV, by

administering (A) to the animal. WIDER DISCLOSURE - Disclosed are

pharmaceutical compositions for prophylactically or therapeutically

treating CMV infections. BIOTECHNOLOGY - Preparation: (I) is produced

by standard recombinant techniques. Preferred Method: M1 involves

contacting a chemokine with US28, US28 homolog, its fragment or

variant, or a cell expressing the above said compounds, in the presence

of (A) and determining whether (A) inhibits the binding between the chemokine and US28, its homolog, fragment or variant. The (A) is an antibody that specifically binds to (I), or a small molecule. The cell is infected with CMV or transfected with a heterologous nucleic acid encoding US28, its homolog, fragment or variant. The protein comprises at least 10 contiguous amino acids of S1 and binds to the chemokine. The method further involves administering (A) to a non-human animal infected with CMV and determining whether (A) inhibits the dissemination of CMV from a primary site of infection in non-human animal such as primate e.g. rhesus monkey. The CMV is rhCMV. The method further involves determining whether viral titer in a saliva, urine or blood sample obtained from the non-human animal is detectably less than viral titer in a corresponding sample obtained from a control animal.

The method further involves obtaining a peripheral blood sample from a non-human animal, amplifying a region of CMV which is present in the sample with a set of primers that specifically hybridize to a set of CMV genome to form an amplified product, and detecting the amplified product. The method involves obtaining and staining a tissue sample of a non-human animal with an antibody that specifically binds to CMV.

Alternately, activated T cells and/or memory cells in a peripheral blood sample taken from the non-human animal are detected. In M2, (A) interferes with the expression of a target nucleic acid encoding (I) in cells of the animal. The interference is achieved by administering an antisense nucleic acid that specifically hybridizes to a target nucleic acid or ribozyme that specifically recognizes the target nucleic acid.

The target nucleic acid encodes US28, human UL33 or human UL78. The (A) is a vaccine which generates an immune response in an animal, where the

vaccine is attenuated through inhibition of expression or activity of

US28 or US28 homolog. The vaccine comprises an immunogenic human

cytomegalovirus (HCMV) polypeptide encoded by at least a region of HCMV

genome in which the polynucleotide segment US28 or UL33 or UL78 has

been inactivated. The CMV titer is reduced by 5-fold or greater as

measured in blood, saliva or urine, following administration of (A).

The interference results in a delay in appearance or reduction of

levels of reactive leucocyte in the peripheral blood of the animal.

Preferred Sequence: (I) is encoded by a nucleic acid segment that

hybridizes under stringent conditions to a sequence comprising 1085,

990, 1019, 991, 1460, 1150, 996 or 1339 nucleotides fully defined in

the specification. The immunogenic CMV polypeptide in (III) is an HCMV

polypeptide encoded by at least a region of the HCMV genome. ACTIVITY -

Vincide; neuroprotective; antiinflammatory; ophthalmological.

MECHANISM OF ACTION - Inhibitor of CMV dissemination; inhibitor of

binding of chemokine to US28; vaccine (claimed). No suitable data

given. USE - (III) is useful for treating an animal infected with

cytomegalovirus or at a risk of infection by CMV. (III) is also useful

for inducing a therapeutic or protective immune response in a patient

and in methods for treating diseases including retinitis, encephalitis

and pneumocystis caused by CMV infection. ADMINISTRATION - (III) is administered through oral, intranasal, intraperitoneal, intravenous, intramuscular, subcutaneous, subdermal or transdermal route. The dosage is 0.05-20 mg/kg, preferably 1-10 mg/kg/day. EXAMPLE - Rhesus dermal fibroblasts (RhDF) were infected with rhesus cytomegalovirus (CMV) strain 68.1. At 96 hours post infection supernatants were collected, and virions were pelleted. The isolated virions were resuspended in TE buffer and proteinase K added to a final concentration of 0.2 microg/ml, sodium dodecyl sulfate to a final concentration of 1% and RNaseA to a final concentration of 10 microg/ml. The resulting mixture was incubated for 2 hours at 65degreesC. Viral DNA was precipitated with ethanol and then subjected to sequencing. The DNA was used to create a shot gun library using hydroshearing and producing inserts of about 3000 nucleotides in length. Individual clones were sequenced using ABI Prism BigDye terminator chemistry. The 220 kb genome was covered to an average accuracy of 6x sequence. Individual reads were assembled into contiguous fragments. Homologs of human CMV genes were elucidated using the basic local alignment search tool X program. Sequence was further analyzed using the BioNavigator bio-informatic program set. Analysis of the rhCMV genome indicated the presence of a number of open reading frames having homology with the human CMV US28 open reading frame. The regions of homology were referred to as rhUS28.1 (comprising 344 nucleotides), rhUS28.2 (comprising 339 nucleotides), rhUS28.3 (comprising 340 nucleotides), rhUS28.4 (comprising 328 nucleotides) and rhUS28.5 (comprising 485 nucleotides). Other regions having homology to human UL33 and human UL78 were also identified and named rhUL33 (comprising 401 nucleotides), rhUL33 spliced (comprising 365 nucleotides) and rhUL78 (comprising 423 nucleotides) (sequences fully defined in the specification). (95 pages)

8/7/108 (Item 13 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

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0260109 DBR Accession No.: 2000-14599

Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination - the use of nucleic acid vaccine for treating disease

AUTHOR: Barouch D H; Santra S; Schmitz J E; Kuroda M J; Fu T M; Wagner W; Biliska M; Craiu A; Zheng X X; Krivulka G R; Beaudry K; Lifton M A; Nickerson C E; Trigona W L; Punt K; Freed D C; Guan L; Dubey S; Casimiro D; Simon A; Davies M E; Chastain M; Strom T B; Gelman R S; Montefiori D C; Lewis M G; Emini E A; Shiver J W; Letvin N L
CORPORATE AFFILIATE: Beth-Israel-Med.Cent.New-York Harvard Med.Sch. CORPORATE SOURCE: Department of medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, 3320 Brookline Avenue, Boston, MA 02215, USA. email: dan barouch@hotmail.com
JOURNAL: Science (290, 5491, 486-92) 2000

ISSN: 0036-8075 CODEN: SCIEAS

LANGUAGE: English

ABSTRACT: Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination was studied. Twenty rhesus monkeys were immunized with a sham pV1R plasmid (n=8), SiVmac239 Gag and HIV virus-1 89.6p Env DNA vaccines plus interleukin (IL)-2/immunoglobulin G (Ig)(n=8). The plasmid DNA vaccines expressed genes optimized for high-level expression and used a cytomegalo virus promoter in a pV1R vector backbone. The IL-2/Ig was administered as purified human IL-2/Ig protein in four animals. After SHIV-89.6P infection, sham-vaccinated monkeys developed weak cytotoxic T-lymphocyte (CTL) responses rapid loss of CD4+ T-lymphocyte, whereas all monkeys that received plasmid DNA vaccines plus IL-2/Ig had complete preservation of their postchallenge CD4+ . No virus-specific CD4+ T-lymphocyte responses, high set point viral loads, significant clinical disease progression, and death in half of the animals by day 140 after challenge. In contrast, all monkeys that received DNA vaccines augment occurred with IL-2/Ig were infected, but demonstrated potent secondary CTL responses, stable CD4+ T-lymphocyte count and no evidence of clinical disease. (45 ref)

?ts157/1-7

15/7/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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14191899 22212801 PMID: 12224510

Viral chemokine receptors and chemokines in human cytomegalovirus trafficking and interaction with the immune system. CMV chemokine receptors.

Beisser P S; Goh C S; Cohen F E; Michelson S; et al

Unité d'Immunologie Virale, Institut Pasteur, 28 Rue du Docteur Roux, 75274 Paris, France.

Current topics in microbiology and immunology (Germany) 2002, 269

p203-34, ISSN 0070-217X Journal Code: 0110513

Document type: Journal Article; Review; Review; Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The ubiquitous, opportunistic pathogen human cytomegalovirus (CMV) encodes several proteins homologous to those of the host organism. Four different CMV genes encode chemokine receptor-like peptides. These genes, UL33, UL78, US27, and US28, are expressed at various stages of infection in vitro. Their functions remain largely unknown. To date, chemokine binding and signalling has only been demonstrated for the US28 gene product. Putative ligands for the other CMV-encoded chemokine receptors are discussed on basis of phylogenetic analysis. The potential roles of these receptors in virus trafficking, persistence, and immune evasion are summarized. Similarly, modulation of expression of the host chemokines

IL-8, MCP-1a and RANTES in relation to viral dissemination and persistence is reviewed. (117 Refs.)

Record Date Created: 20020912

15/7/2 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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14141187 22343858 PMID: 12455411

The HCMV chemokine receptor US28 is a potential target in vascular disease.

Streblow D N; Orloff S L; Nelson J A; et al

Department of Molecular Microbiology and Immunology, Vaccine and Gene Therapy Institute, Oregon Health Sciences University, Portland, OR 97201, USA.

Curr Drug Targets Infect Disord (Netherlands) Aug 2001, 1 (2) p151-8, ISSN 1568-0053 Journal Code: 101128002

Contract/Grant No.: AI 21640; AI; NIAID; HL 65754; HL; NHLBI; +

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The human cytomegalovirus (HCMV) has been implicated in the acceleration of vascular disease for some time. The development of vascular disease involves a chronic inflammatory process with many contributing factors, and of these, chemokines and their receptors have recently been identified as key mediators. Interestingly, HCMV encodes four potential chemokine receptors (US27, US28, UL33 and UL78). Of these virally-encoded chemokine receptors, US28 has been the most widely characterized. US28 binds many of the CC-chemokines, and this class of chemokines contributes to the development of vascular disease. Importantly, HCMV infection mediates in vitro SMC migration, which is dependent upon expression of US28 and CC-chemokine binding. US28 and the US28 functional homologues that are capable of inducing the migration of SMC represent potential targets in the treatment of CMV-accelerated vascular disease such as atherosclerosis, restenosis, and transplant vascular sclerosis. (80 Refs.)

Record Date Created: 20021128

15/7/3 (Item 3 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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13494721 22129235 PMID: 12134021

Murine cytomegalovirus (CMV) M33 and human CMV US28 receptors exhibit similar constitutive signaling activities.

Walshoe Maria; Kiedal Thomas N; Farrell Helen; Schwartz Thue W

Laboratory for Molecular Pharmacology, The Panum Institute, University of Copenhagen, United Kingdom.

Journal of virology (United States) Aug 2002, 76 (16) p8161-8,

ISSN 0022-538X Journal Code: 0113724

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Cellular infection by cytomegalovirus (CMV) is associated with very early G-protein-mediated signal transduction and reprogramming of gene expression. Here we investigated the involvement of human CMV (HCMV)-encoded US27, US28, and UL33 receptors as well as murine CMV-encoded M33 transmembrane (TTM) receptors in host cell signaling mechanisms. HCMV-encoded US27 did not show any constitutive activity in any of the studied signaling pathways; in contrast, US28 and M33 displayed ligand-independent, constitutive signaling through the G protein q (Gq)/phospholipase C pathway. In addition, M33 and US28 also activated the transcription factor NF-kappaB as well as the cyclic AMP response element binding protein (CREB) in a ligand-independent, constitutive manner. The use of specific inhibitors indicated that the p38 mitogen-activated protein (MAP) kinase but not the extracellular signal-regulated kinase 1/2-MAP kinase pathway is involved in M33- and US28-mediated CREB activation but not NF-kappaB activation. Interestingly, UL33-the HCMV-encoded structural homologue of M33-was only marginally constitutively active in the Gq/phospholipase C turnover and CREB activation assays and did not show any constitutive activity in the NF-kappaB pathway, where M33 and US28 were highly active. Hence, CMVs appear to have conserved mechanisms for regulating host gene transcription, i.e., constitutive activation of certain kinases and transcription factors through the constitutive activities of TTM proteins. These data, together with the previous identification of the incorporation of such proteins in the viral envelope, suggest that these proteins could be involved in the very early reprogramming of the host cell during viral infection.

Record Date Created: 20020722

15/7/4 (Item 4 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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13436913 21884601 PMID: 11886592

Localization of HCMV UL33 and US27 in endocytic compartments and viral membranes.

Fraile-Ramos Alberto; Pelchen-Matthews Annegret; Kiedal Thomas N; Browne Helena; Schwartz Thue W; Marsh Mark

Department of Biochemistry and Molecular Biology, Cell Biology Unit, MRC Laboratory for Molecular Cell Biology, University College London, Gower Street, London WC1E 6BT, UK.

Traffic (Copenhagen, Denmark) (Denmark) Mar 2002, 3 (3) p218-32, ISSN 1398-9219 Journal Code: 100939340

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The human cytomegalovirus genome encodes four putative seven transmembrane domain chemokine receptor-like proteins. Although important in viral pathogenesis, little is known about the properties or functions of these proteins. We previously reported that US28 is located in endocytic vesicles and undergoes constitutive endocytosis and recycling. Here we studied the cellular distributions and trafficking of two other human cytomegalovirus chemokine receptor-like proteins, UL33 and US27, in transfected and human cytomegalovirus-infected cells. Immunofluorescence staining indicated that UL33 and US27 are located at the cell surface, although the majority of both proteins was seen in intracellular organelles located in the perinuclear region of the cell. The intracellular pools of UL33 and US27 showed overlap with markers for endocytic organelles. Antibody-feeding experiments indicated that cell surface US27 undergoes endocytosis. By immunogold labeling of cryosections and electron microscopy, UL33 was seen to localize to multivesicular bodies (MVBs or multivesicular endosomes). Electron microscopy analysis of human cytomegalovirus-infected cells showed that most virus particles wrapped individually into short membrane cisternae, although virus particles were also occasionally seen within and budding into MVBs. Electron microscopy immunolocalization of viral UL33 and US27 on ultrathin cryosections of human cytomegalovirus-infected cells showed gold particles over the membranes into which virions were wrapping, in small membrane tubules and vesicles and in MVBs. Labeling of the human cytomegalovirus glycoproteins gB and gH indicated that these proteins were also present in the same membrane structures. This first electron microscopy analysis of human cytomegalovirus assembly using immunolabeling suggests that the localization of UL33, US27 and US28 to endosomes may allow these proteins to be incorporated into the viral membrane during the final stages of human cytomegalovirus assembly.

Record Date Created: 20020311

15/7/5 (Item 5 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09956628 98406221 PMID: 9733857

Functional analysis of the human cytomegalovirus US28 gene by insertion mutagenesis with the green fluorescent protein gene.

Vieira J, Schall T J, Corey L, Geballe A P

Department of Laboratory Medicine, University of Washington, Seattle, Washington 98195, USA. vieiraj@u.washington.edu

Journal of virology (UNITED STATES) Oct 1998, 72 (10) p8158-65, ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: AI26672; AI; NIAID

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The protein encoded by the US28 gene of human cytomegalovirus (HCMV) has homology to G protein-coupled receptors (GCR). Previous studies demonstrated that recombinant US28 protein can bind the beta class of chemokines (K. Neote, D. DiGregorio, J. Y. Mak, R. Horuk, and T. J. Schall, Cell 72:415-425, 1993) and induce a rise in intracellular calcium after the binding of chemokines (J. L. Gao and P. M. Murphy, J. Biol. Chem. 269:28539-28542, 1994). In order to investigate the function of the US28 protein in virus-infected cells, a recombinant HCMV (HV5.8) was constructed, with the US28 open reading frame disrupted by the insertion of the *Escherichia coli* gpt gene and the gene for the green fluorescent protein. The US28 gene is not required for growth in human fibroblasts (HF). HF infected with wild-type HCMV bound RANTES at 24 h postinfection and demonstrated an intracellular calcium flux induced by RANTES. In cells infected with HV5.8, RANTES did not bind or induce a calcium flux, demonstrating that US28 is responsible for the beta-chemokine binding and induced calcium signaling in HCMV-infected cells. The ability of the US28 gene to bind chemokines was shown to cause a significant reduction in the concentration of RANTES in the medium of infected cells. Northern analysis of RNA from infected cells showed that US28 is an early gene, while US27 (another GCR) is a late gene.

Record Date Created: 19981007

15/7/6 (Item 6 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09951955 98401020 PMID: 9730887

Chemokine sequestration by viral chemoreceptors as a novel viral escape strategy: withdrawal of chemokines from the environment of cytomegalovirus-infected cells.

Bodaghi B, Jones T R, Zipeto D, Vita C, Sun L, Laurent L,

Arenzana-Seisdedos F, Virélizier J L, Michelson S

Unité d'Immunologie Virale, Institut Pasteur, Paris, France.

Journal of experimental medicine (UNITED STATES) Sep 7 1998, 188 (5) p855-66, ISSN 0022-1007 Journal Code: 2985109R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human cytomegalovirus (HCMV), a betaherpesvirus, has developed several ways to evade the immune system, notably downregulation of cell surface expression of major histocompatibility complex class I heavy chains. Here we report that HCMV has devised another means to compromise immune surveillance mechanisms. Extracellular accumulation of both constitutively produced monocyte chemoattractant protein (MCP)-1 and tumor necrosis factor-superinduced RANTES (regulated on activation, normal T cell

expressed and secreted) was downregulated in HCMV-infected fibroblasts in the absence of transcriptional repression or the expression of polyadenylated RNA for the cellular chemokine receptors CCR-1, CCR-3, and CCR-5. Competitive binding experiments demonstrated that HCMV-infected cells bind RANTES, MCP-1, macrophage inflammatory protein (MIP)-1beta, and MCP-3, but not MCP-2, to the same receptor as does MIP-1alpha, which is not expressed in uninfected cells. HCMV encodes three proteins with homology to CC chemokine receptors: US27, US28, and UL33. Cells infected with HCMV mutants deleted of US28, or both US27 and US28 genes, failed to downregulate extracellular accumulation of either RANTES or MCP-1. In contrast, cells infected with a mutant deleted of US27 continues to bind and downregulate those chemokines. Depletion of chemokines from the culture medium was at least partially due to continuous internalization of extracellular chemokine, since exogenously added, biotinylated RANTES accumulated in HCMV-infected cells. Thus, HCMV can modify the chemokine environment of infected cells through intense sequestering of CC chemokines, mediated principally by expression of the US28-encoded chemokine receptor.

Record Date Created: 19980924

15777 (Item 7 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

09169206 97076221 PMID: 8918538

Identification of the human cytomegalovirus G protein-coupled receptor homologue encoded by UL33 in infected cells and enveloped virus particles.

Margulies B J; Browne H; Gibson W

Retrovirus Biology Laboratory, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.

Virology (UNITED STATES) Nov 1 1996, 225 (1) p111-25, ISSN 0042-6822 Journal Code: 0110674

Contract/Grant No.: AI13718; AI; NIAID; AI22711; AI; NIAID; GM07445; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human cytomegalovirus (HCMV), strain AD169, contains four genes (US27, US28, UL33, and UL78) that encode putative homologues of cellular G protein-coupled receptors (GCRs). GCRs transduce extracellular signals to alter intracellular processes, and there is evidence that HCMV may elicit such changes at early times following infection. The US27, US28, and UL33 genes are transcribed during infection, and the US28 gene product has been found to be a functional receptor for the beta-chemokine class of immune modulators. The US27, UL33, and UL78 gene products have not been described and we have concentrated on identifying the UL33 protein because it is the most highly conserved of the GCR homologues among the human beta and gamma

herpesviruses. We report here cloning UL33 into a recombinant baculovirus (rBV) and expressing it in insect cells; constructing a mutant HCMV with a disrupted UL33 gene; and identifying the UL33 protein in HCMV-infected cells and virus particles. Our results demonstrate that the UL33 protein (i) is expressed as a approximately 36-kDa, heat-aggregatable protein in rBV-infected cells, (ii) is modified heterogeneously by asparagine-linked glycosylation and expressed as a > or = 58-kDa glycoprotein that is present in the region of the cytoplasmic inclusions in HCMV-infected fibroblasts, (iii) is present in virions and two other enveloped virus particles, and (iv) is not essential for growth of HCMV in human foreskin fibroblast cultures.

Record Date Created: 19961223

?ts1677/1 2

1677/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

14343047 22418707 PMID: 12530329

Virally encoded chemokines and chemokine receptors in the role of viral infections.

Holst Peter J; Luitichau Hans R; Schwartz Thue W; Rosenkilde Mette M; et al

Laboratory for Molecular Pharmacology, Department of Pharmacology, Panum Institute, Copenhagen, Denmark.

Contributions to microbiology (Switzerland) 2003, 10 p232-52, ISSN 1420-9519 Journal Code: 9815689

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: In Process

Large DNA viruses such as pox- and in particular herpesviruses are notorious in their ability to evade the immune system and to be maintained in the general population. Based on the accumulated knowledge reviewed in this study it is evident that important mechanisms of these actions are the acquisition and modification of host-encoded chemokines and chemokine receptors. The described viral molecules leave nothing to chance and have thoroughly and efficiently corrupted the host immune system. Through this process viruses have identified key molecules in antiviral responses by their inhibition of these or potent ways to alter an efficient antiviral response to a weak Th2-driven response. Examples here are the chemokine scavenging by US28, attraction of Th2 cells and regulatory cells by vMMP-3 and the selective engaging of CCR8 by MC148. Important insights into viral pathology and possible targets for antiviral therapies have been provided by UL33, UL78 and in particular ORF74 and the chances are that many more will follow. In HHV8 vMMP-2 and the chemokine-binding proteins potent anti-inflammatory agents have been provided. These have already had their potential demonstrated in animal models and may in their native or modified forms represent useful therapies in humans.

Record Date Created: 20030117

16/7/2 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

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0291312 DBR Accession No.: 2002-13159 PATENT

Isolated or recombinant homologs of US28 proteins and nucleic acids

encoding the proteins, for use in vaccine compositions for treating an

animal infected with, or at risk of infection by, cytomegalovirus -

vector expression in host cell for recombinant protein gene production,

antibody, transgenic animal, sense, antisense oligonucleotide, ribozyme

useful in virus infection gene therapy and vaccine

AUTHOR: SCHALL T J; PENFOLD M

PATENT ASSIGNEE: CHEMOCENTRIX INC 2002

PATENT NUMBER: WO 200218954 PATENT DATE: 20020307 WPI ACCESSION

NO.:

2002-351718 (200238)

PRIORITY APPLIC. NO.: US 229365 APPLIC. DATE: 20000830

NATIONAL APPLIC. NO.: WO 2001US27392 APPLIC. DATE: 20010830

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated or recombinant

homolog of

US28 protein (I) which binds a chemokine (encoded by an open reading frame in the unique short (US) region in human cytomegalovirus (CMV) genome), having at least 75% identity to a sequence (S1) fully defined in the specification, over a region of at least 40 amino acids in length, or a protein comprising at least 12 amino acids of S1, is new.

DETAILED DESCRIPTION - S1 comprises a sequence of 344, 339, 340, 328, 485, 401, 365 or 423 amino acids fully defined in the specification

(amino acid sequences of rhesus monkey proteins RhUS28.1, RhUS28.2,

RhUS28.3. RhUS28.4, RhUS28.5, RhUL78 (encoded by an open reading frame

78 in the unique long (UL) region of CMV genome), RhUL33 and RhUL33

spliced, respectively). INDEPENDENT CLAIMS are also included for the

following: (1) an isolated, purified or recombinant nucleic acid (II)

encoding (I) (US28 homolog); (2) a vector comprising (II); (3) a cell

comprising (II); (4) a vaccine (III) comprising an immunogenic CMV

polypeptide encoded by at least a region of CMV genome in which the

polynucleotide sequence encoding US28 or its homolog has been

inactivated; (5) identifying (M1) an agent that reduces CMV

dissemination in an animal, by determining whether the agent (A)

inhibits the expression or activity of US28 or US28 homolog, or

fragment or variant of US28 or US28 homolog; and (6) treating (M2) an

animal infected with CMV or at risk of infection by CMV, by

administering (A) to the animal. WIDER DISCLOSURE - Disclosed are

pharmaceutical compositions for prophylactically or therapeutically

treating CMV infections. BIOTECHNOLOGY - Preparation: (I) is produced

by standard recombinant techniques. Preferred Method: M1 involves

contacting a chemokine with US28, US28 homolog, its fragment or variant, or a cell expressing the above said compounds, in the presence of (A) and determining whether (A) inhibits the binding between the chemokine and US28, its homolog, fragment or variant. The (A) is an antibody that specifically binds to (I), or a small molecule. The cell is infected with CMV or transfected with a heterologous nucleic acid encoding US28, its homolog, fragment or variant. The protein comprises at least 10 contiguous amino acids of S1 and binds to the chemokine. The method further involves administering (A) to a non-human animal infected with CMV and determining whether (A) inhibits the dissemination of CMV from a primary site of infection in non-human animal such as primate e.g. rhesus monkey. The CMV is rhCMV. The method further involves determining whether viral titer in a saliva, urine or blood sample obtained from the non-human animal is detectably less than viral titer in a corresponding sample obtained from a control animal.

The method further involves obtaining a peripheral blood sample from a non-human animal, amplifying a region of CMV which is present in the sample with a set of primers that specifically hybridize to a set of CMV genome to form an amplified product, and detecting the amplified product. The method involves obtaining and staining a tissue sample of a non-human animal with an antibody that specifically binds to CMV.

Alternately, activated T cells and/or memory cells in a peripheral blood sample taken from the non-human animal are detected. In M2, (A) interferes with the expression of a target nucleic acid encoding (I) in cells of the animal. The interference is achieved by administering an antisense nucleic acid that specifically hybridizes to a target nucleic acid or ribozyme that specifically recognizes the target nucleic acid.

The target nucleic acid encodes US28, human UL33 or human UL78. The (A) is a vaccine which generates an immune response in an animal, where the

vaccine is attenuated through inhibition of expression or activity of

US28 or US28 homolog. The vaccine comprises an immunogenic human

cytomegalovirus (HCMV) polypeptide encoded by at least a region of HCMV

genome in which the polynucleotide segment US28 or UL33 or UL78 has

been inactivated. The CMV titer is reduced by 5-fold or greater as

measured in blood, saliva or urine, following administration of (A).

The interference results in a delay in appearance or reduction of

levels of reactive leucocyte in the peripheral blood of the animal.

Preferred Sequence: (I) is encoded by a nucleic acid segment that

hybridizes under stringent conditions to a sequence comprising 1085,

990, 1019, 991, 1460, 1150, 996 or 1339 nucleotides fully defined in

the specification. The immunogenic CMV polypeptide in (II) is an HCMV

polypeptide encoded by at least a region of the HCMV genome. ACTIVITY -

Vincide; neuroprotective; antiinflammatory; ophthalmological.

MECHANISM OF ACTION - Inhibitor of CMV dissemination; inhibitor of

binding of chemokine to US28; vaccine (claimed). No suitable data

given. USE - (III) is useful for treating an animal infected with

cytomegalovirus or at a risk of infection by CMV. (III) is also useful

for inducing a therapeutic or protective immune response in a patient and in methods for treating diseases including retinitis, encephalitis and pneumocystis caused by CMV infection. ADMINISTRATION - (III) is administered through oral, intranasal, intraperitoneal, intravenous, intramuscular, subcutaneous, subdermal or transdermal route. The dosage is 0.05-20 mg/kg, preferably 1-10 mg/kg/day. EXAMPLE - Rhesus dermal fibroblasts (RhDF) were infected with rhesus cytomegalovirus (CMV) strain 68.1. At 96 hours post infection supernatants were collected, and virions were pelleted. The isolated virions were resuspended in TE buffer and proteinase K added to a final concentration of 0.2 microg/ml, sodium dodecyl sulfate to a final concentration of 1% and RNaseA to a final concentration of 10 microg/ml. The resulting mixture was incubated for 2 hours at 65degreesC. Viral DNA was precipitated with ethanol and then subjected to sequencing. The DNA was used to create a shot gun library using hydroshearing and producing inserts of about 3000 nucleotides in length. Individual clones were sequenced using ABI Prism BigDye terminator chemistry. The 220 kb genome was covered to an average accuracy of 6x sequence. Individual reads were assembled into contiguous fragments. Homologs of human CMV genes were elucidated using the basic local alignment search tool X program.

Sequence was further analyzed using the BioNavigator bio-informatic program set. Analysis of the rhCMV genome indicated the presence of a number of open reading frames having homology with the human CMV US28 open reading frame. The regions of homology were referred to as rhUS28.1 (comprising 344 nucleotides), rhUS28.2 (comprising 339 nucleotides), rhUS28.3 (comprising 340 nucleotides), rhUS28.4 (comprising 328 nucleotides) and rhUS28.5 (comprising 485 nucleotides). Other regions having homology to human UL33 and human UL78 were also identified and named rhUL33 (comprising 401 nucleotides), rhUL33 spliced (comprising 365 nucleotides) and rhUL78 (comprising 423 nucleotides) (sequences fully defined in the specification). (95 pages)

71 s18/7/24 40 42 43 45 49 52
18/7/24 (Item 24 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.
10803527 20351362 PMID: 10891288

Chemokine receptor-related genetic sequences in an african green monkey simian cytomegalovirus-derived stealth virus.

Martin W J

Center for Complex Infectious Diseases, Rosemead, California, 91770, USA.
Experimental and molecular pathology (UNITED STATES) Aug 2000, 69 (1)
p10-6, ISSN 0014-4800 Journal Code: 0370711

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The US28 gene of human cytomegalovirus (HCMV) codes a cell surface

receptor for both beta chemokine and fractalkine molecules. This receptor facilitates HCMV-induced cell fusion and virus dissemination and influences susceptibility to infection with other viruses, including the human immunodeficiency virus. Five adjacent but divergent open reading frames that potentially code for molecules related to the US28 protein of HCMV are present in an African green monkey simian cytomegalovirus-derived stealth virus. This finding implies a role for chemokines in the pathogenicity of at least some stealth-adapted viruses. It may also help explain the apparent therapeutic benefit achieved in certain stealth virus-infected patients treated with agents that downregulate chemokine production. Copyright 2000 Academic Press.

Record Date Created: 20000803

18/7/40 (Item 40 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.
09890531 98325151 PMID: 9658079

The cytomegalovirus-encoded chemokine receptor US28 can enhance cell-cell fusion mediated by different viral proteins.

Pleskoff O; Treboute C; Alizon M

INSERM U 332, Institut Cochin de Genetique Moleculaire, 75014 Paris, France.

Journal of virology (UNITED STATES) Aug 1998, 72 (8) p6389-97,
ISSN 0022-538X Journal Code: 0113724

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The human cytomegalovirus (CMV) US28 gene encodes a functional CC chemokine receptor. However, this activity was observed in cells transfected to express US28 and might not correspond to the actual role of the protein in the CMV life cycle. Expression of US28 allows human immunodeficiency virus type 1 (HIV-1) entry into certain CD4(+) cells and their fusion with cells expressing HIV-1 envelope (Env) proteins. Such properties were initially reported for the cellular chemokine receptors CCR5 and CXCR4, which behave as CD4-associated HIV-1 coreceptors. We found that coexpression of US28 and either CXCR4 or CCR5 in CD4(+) cells resulted in enhanced syncytium formation with HIV-1 Env+ cells. This positive effect of US28 on cell fusion seems to be distinct from its HIV-1 coreceptor activity. Indeed, enhancement of cell fusion was also observed when US28 was expressed on the HIV-1 Env+ cells instead of an CD4(+) target cells. Furthermore, US28 could enhance cell fusion mediated by other viral proteins, in particular, the G protein of vesicular stomatitis virus (VSV-G). The HIV-1 coreceptor and fusion-enhancing activities could be affected by mutations in different domains of US28. The fusion-enhancing activity of US28 seems to be cell type dependent. Indeed, cells coexpressing VSV-G and US28 fused more efficiently with human, simian, or

feline target cells, while US28 had no apparent effect on fusion with the three mouse or rat cell lines tested. The positive effect of US28 on cell fusion might therefore require its interaction with a cell-specific factor. We discuss a possible role for US28 in the fusion of the CMV envelope with target cells and CMV entry.

Record Date Created: 19980805

18/7/42 (Item 42 from file: 155)

DIALOG(R)/File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

09856286 98285708 PMID: 9621010

Intracellular signaling by the chemokine receptor US28 during human cytomegalovirus infection.

Bilstrom M A, Johnson G L, Avdi N J, Worthen G S

Department of Medicine, National Jewish Medical and Research Center, University of Colorado School of Medicine, Denver, Colorado 80206, USA.

bilstroms@njc.org

Journal of virology (UNITED STATES) Jul 1998, 72 (7) p5535-44,

ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: GM-30324; GM; NIGMS; HL-34303; HL; NHLBI; HL-40784;

HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In patients with impaired cell-mediated immune responses (e.g., lung transplant recipients and AIDS patients), cytomegalovirus (CMV) infection causes severe disease such as pneumonitis. However, although immunocompetency in the host can protect from CMV disease, the virus persists by evading the host immune defenses. A model of CMV infection of the endothelium has been developed in which inflammatory stimuli, such as the CC chemokine RANTES, bind to the endothelial cell surface, stimulating calcium flux during late times of CMV infection. At 96 h postinfection, CMV-infected cells express mRNA of the CMV-encoded CC chemokine receptor US28 but do not express mRNA of other CC chemokine receptors that bind RANTES (CCR1, CCR4, CCR5). Cloning and stable expression of the receptor CMV US28 in human kidney epithelial cells (293 cells) with and without the heterotrimeric G protein alpha16 indicated that CMV US28 couples to both Galphai and Galpha16 proteins to activate calcium flux in response to the chemokines RANTES and MCP-3. Furthermore, cells that coexpress US28 and Galpha16 responded to RANTES stimulation with activation of extracellular signal-regulated kinase, which could be attributed, in part, to specific Galpha16 coupling. Thus, through expression of the CC chemokine receptor US28, CMV may utilize resident G proteins of the infected cell to manipulate cellular responses stimulated by chemokines.

Record Date Created: 19980701

18/7/43 (Item 43 from file: 155)

DIALOG(R)/File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

09625698 98037607 PMID: 9371556

Utilization of chemokine receptors, orphan receptors, and herpesvirus-encoded receptors by diverse human and simian immunodeficiency viruses.

Rucker J, Edinger A L, Sharron M, Samson M, Lee B, Berson J F, Yi Y,

Margulies B, Collman R G, Doranz B J, Parmentier M, Doms R W

Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104, USA.

Journal of virology (UNITED STATES) Dec 1997, 71 (12) p8999-9007, ISSN 0022-538X Journal Code: 0113724

Contract/Grant No.: AI-35383; AI; NIAID; AI-38225; AI; NIAID; AI-40880;

AI; NIAID; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human immunodeficiency virus type 1 (HIV-1) requires both CD4 and a coreceptor to infect cells. Macrophage-tropic (M-tropic) HIV-1 strains utilize the chemokine receptor CCR5 in conjunction with CD4 to infect cells, while T-cell-tropic (T-tropic) strains generally utilize CXCR4 as a coreceptor. Some viruses can use both CCR5 and CXCR4 for virus entry (i.e., are dual-tropic), while other chemokine receptors can be used by a subset of virus strains. Due to the genetic diversity of HIV-1, HIV-2, and simian immunodeficiency virus (SIV) and the potential for chemokine receptors other than CCR5 or CXCR4 to influence viral pathogenesis, we tested a panel of 28 HIV-1, HIV-2, and SIV envelope (Env) proteins for the ability to utilize chemokine receptors, orphan receptors, and herpesvirus-encoded chemokine receptor homologs by membrane fusion and virus infection assays. While all Env proteins used either CCR5 or CXCR4 or both, several also used CCR3. Use of CCR3 was strongly dependent on its surface expression levels, with a larger number of viral Env proteins being able to utilize this coreceptor at the higher levels of surface expression. ChemR1, an orphan receptor recently shown to bind the CC chemokine 1309 (and therefore renamed CCR8), was expressed in monocyte and lymphocyte cell populations and functioned as a coreceptor for diverse HIV-1, HIV-2, and SIV Env proteins. Use of ChemR1/CCR8 by SIV strains was dependent in part on V3 loop sequences. The orphan receptor V28 supported Env-mediated cell-cell fusion by four T- or dual-tropic HIV-1 and HIV-2 strains. Three additional orphan receptors failed to function for any of the 28 Env proteins tested. Likewise, five of six seven-transmembrane-domain receptors encoded by herpesviruses did not support Env-mediated membrane fusion. However, the chemokine receptor US28, encoded by cytomegalovirus, did support inefficient infection by two HIV-1 strains. These findings indicate that additional chemokine receptors can function as HIV and SIV coreceptors and

that surface expression levels can strongly influence coreceptor use.

Record Date Created: 19971224

18/7/45 (Item 45 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09433310 97334446 PMID: 9188536

Identification of a chemokine receptor encoded by human cytomegalovirus as a cofactor for HIV-1 entry.

Pleskoff O, Treboule C, Brelot A, Heveker N, Senan M, Alizon M

Inserm U.332, Institut Cochin de Genetique Moleculaire, 22 rue Mechain, 75014 Paris, France.

Science (UNITED STATES) Jun 20 1997, 276 (5320) p1874-8, ISSN

0036-8075 Journal Code: 0404511

Comment in Science. 1997 Jun 20;276(5320) 1794; Comment in PMID 9206839

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The human cytomegalovirus encodes a beta-chemokine receptor (US28) that is distantly related to the human chemokine receptors CCR5 and CXCR4, which also serve as cofactors for the entry into cells of human immunodeficiency virus-type 1 (HIV-1). Like CCR5, US28 allowed infection of CD4-positive human cell lines by primary isolates of HIV-1 and HIV-2, as well as fusion of these cell lines with cells expressing the viral envelope proteins. In addition, US28 mediated infection by cell line-adapted HIV-1 for which CXCR4 was an entry cofactor.

Record Date Created: 19970701

18/7/49 (Item 49 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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08539383 95298042 PMID: 7540006

The cytomegalovirus US28 protein binds multiple CC chemokines with high affinity.

Kuhn D E, Beall C J, Kolattukudy P E

Ohio State University, Neurobiotechnology Center, Columbus 43210, USA.

Biochemical and biophysical research communications (UNITED STATES) Jun 6 1995, 211 (1) p325-30, ISSN 0006-291X Journal Code: 0372516

Contract/Grant No.: HL48916; HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human cytomegalovirus encodes several proteins with high similarity to seven transmembrane domain receptors. We investigated the ability of one of these proteins, the product of the US28 open reading frame, to bind various

chemoattractant ligands. When transfected into COS-7 cells, the US28 product conferred high affinity binding to the labeled chemokines monocyte chemoattractant protein-1 (MCP-1) ($K_d = 6.0 \times 10^{-10}$ M) and RANTES ($K_d = 2.7 \times 10^{-10}$ M). Binding of these labeled ligands could be competed by the unlabeled macrophage inflammatory proteins MIP-1 alpha and MIP-1 beta, with K_d values in the range 1.2×10^{-9} to 7.5×10^{-9} M. Comparisons of the sequences of US28 and other receptors that bind chemokines should help to define regions responsible for receptor-ligand interactions.

Record Date Created: 19950710

18/7/52 (Item 52 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

08292641 95050648 PMID: 7961796

Human cytomegalovirus open reading frame US28 encodes a functional beta chemokine receptor.

Gao J L, Murphy P M

Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892.

Journal of biological chemistry (UNITED STATES) Nov 18 1994, 269 (46) p28539-42, ISSN 0021-9258 Journal Code: 2985121R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Human cytomegalovirus infects epithelial, smooth muscle, and white blood cells in vivo causing acute, latent, and chronic infections. A data base search revealed that the amino acid sequence of the putative protein encoded by open reading frame US28 of human cytomegalovirus is approximately 30% identical to those of the mammalian leukocyte receptors for alpha and beta chemokines. This suggested that US28 was originally copied from a human chemokine receptor gene, perhaps to provide the virus with a selective advantage through molecular mimicry. Chemokines regulate the trafficking and activation of mammalian leukocytes and activate calcium-mobilizing, heptahelical, G protein-coupled receptors. We now show that US28 encodes a promiscuous calcium-mobilizing receptor for the beta chemokines RANTES (regulated upon activation, normal T expressed and secreted), macrophage inflammatory protein-1 alpha, and monocyte chemoattractant protein-1, but not for the alpha chemokines interleukin-8 or gamma IP10. The chemokine selectivity of the US28 product is distinct from that of known mammalian beta chemokine receptors. This finding suggests a role for beta chemokines in the pathogenesis of human cytomegalovirus infection by transmembrane signaling via the product of US28.

Record Date Created: 19941219

941977/17 21 30 33 35

1977/17 (Item 17 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

10583547 20107284 PMID: 10639317

Characterization of the U(L)33 gene product of herpes simplex virus 1.

Reynolds A E; Fan Y; Baines J D

Department of Microbiology, Cornell University, Ithaca, New York, 14853, USA.

Virology (UNITED STATES) Jan 20 2000, 266 (2) p310-8, ISSN

0042-6822 Journal Code: 0110674

Contract/Grant No.: F32-GM20448; GM; NIGMS; RO1-GM50740; GM; NIGMS;

T32-RR07060; RR; NCRR

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The U(L)33 protein is one of six genes (including U(L)6, U(L)15, U(L)17, U(L)28, and U(L)32) required for cleavage of viral concatemeric DNA into unit-length genomes and packaging of the virus genomes into preformed capsids. The U(L)25 gene product is dispensable for cleavage of viral DNA but essential for packaging of DNA into capsids. A polyclonal antiserum was produced against an affinity-purified protein containing the full-length U(L)33 gene product of herpes simplex virus 1 fused to glutathione-S-transferase. A protein of approximate M(r) 19,000 that reacted with the antiserum was detected in immunoblots of herpes simplex virus 1-infected cellular lysates. This protein was not detected in lysates of mock-infected cells or cells infected with a mutant virus containing a stop codon in U(L)33, indicating that the 19,000 M(r) protein is the product of the U(L)33 open reading frame. The U(L)33 gene product was not detected in purified virions or capsids. Accumulation of the U(L)33 protein to detectable levels required viral DNA synthesis, indicating that the protein was regulated as a late gene. Indirect immunofluorescence analysis demonstrated that U(L)33 protein accumulated predominantly within replication compartments in the central domains of infected cell nuclei and within the cytoplasm. Localization of the U(L)33 gene product in replication compartments was maintained in cells infected with a variety of cleavage/packaging mutants. Copyright 2000 Academic Press.

Record Date Created: 20000229

1977/21 (Item 21 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

(c) format only 2003 The Dialog Corp. All rts. reserv.

10373667 99370163 PMID: 10438809

Deletion of the R78 G protein-coupled receptor gene from rat cytomegalovirus results in an attenuated, syncytium-inducing mutant strain.

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The rat cytomegalovirus (RCMV) R78 gene belongs to an uncharacterized class of viral G protein-coupled receptor (GCR) genes. The predicted amino acid sequence of the R78 open reading frame (ORF) shows 25 and 20% similarity with the gene products of murine cytomegalovirus M78 and human cytomegalovirus UL78, respectively. The R78 gene is transcribed throughout the early and late phases of infection in rat embryo fibroblasts (REF) in vitro. Transcription of R78 was found to result in three different mRNAs:

(i) a 1.8-kb mRNA containing the R78 sequence, (ii) a 3.7-kb mRNA containing both R77 and R78 sequences, and (iii) a 5.7-kb mRNA containing at least ORF R77 and ORF R78 sequences. To investigate the function of the R78 gene, we generated two different recombinant virus strains: an RCMV R78 null mutant (RCMVDeltaR78a) and an RCMV mutant encoding a GCR from which the putative intracellular C terminus has been deleted (RCMVDeltaR78c).

These recombinant viruses replicated with a 10- to 100-fold-lower efficiency than wild-type (wt) virus in vitro. Interestingly, unlike wt virus-infected REF, REF infected with the recombinants develop a syncytium-like appearance. A striking difference between wt and recombinant viruses was also seen in vivo: a considerably higher survival was seen among recombinant virus-infected rats than among RCMV-infected rats. We conclude that the RCMV R78 gene encodes a novel GCR-like polypeptide that plays an important role in both RCMV replication in vitro and the pathogenesis of viral infection in vivo.

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The R33 G protein-coupled receptor gene of rat cytomegalovirus plays an essential role in the pathogenesis of viral infection.

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Journal of virology (UNITED STATES) Mar 1998, 72 (3) p2352-63, ISSN 0022-538X Journal Code: 0113724

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We have identified a rat cytomegalovirus (RCMV) gene that encodes a G-protein-coupled receptor (GCR) homolog. This gene (R33) belongs to a

family that includes the human cytomegalovirus UL33 gene. R33 was found to be transcribed during the late phase of RCMV infection in rat embryo fibroblasts. Unlike the mRNAs from all the other members of the UL33 family that have been studied to date, the R33 mRNA is not spliced. To study the function of the R33 gene, we constructed an RCMV strain in which the R33 open reading frame is disrupted. The mutant strain (RCMV delatR33) did not show differences in replication from wild-type RCMV upon infection of several rat cell types *in vitro*. However, marked differences were seen between the mutant and wild-type strain in the pathogenesis of infection in immunocompromised rats. First, the mutant strain induced a significantly lower mortality than the wild-type virus did. Second, in contrast to wild-type RCMV, the mutant strain did not efficiently replicate in the salivary gland epithelial cells of immunocompromised rats. Although viral DNA was detected in salivary glands of RCMV delatR33-infected rats up to 14 days postinfection, it could not be detected at later time points. This indicates that although the strain with R33 deleted is probably transported to the salivary glands in a similar fashion to that for wild-type virus, the mutant virus is not able to either enter or replicate in salivary gland epithelial cells. We conclude that the RCMV R33 gene plays a vital role in the pathogenesis of infection.

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09268871 97151142 PMID: 8995678

Identification and characterization of a G protein-coupled receptor homolog encoded by murine cytomegalovirus.

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Journal of virology (UNITED STATES) Feb 1997, 71 (2) p1521-9, ISSN 0022-538X Journal Code: 0113724

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This report describes the identification of a murine cytomegalovirus (MCMV) G protein-coupled receptor (GCR) homolog. This open reading frame (M33) is most closely related to, and collinear with, human cytomegalovirus UL33, and homologs are also present in human herpesvirus 6 and 7 (UL12 for both viruses). Conserved counterparts in the sequenced alpha- or gammaherpesviruses have not been identified to date, suggesting that these genes encode proteins which are important for the biological characteristics of betaherpesviruses. We have detected transcripts for both UL33 and M33 as early as 3 or 4 h postinfection, and these reappear at late

times. In addition, we have identified N-terminal splicing for both the UL33 and M33 RNA transcripts. For both open reading frames, splicing results in the introduction of amino acids which are highly conserved among known GCRs. To characterise the function of the M33 in the natural host, two independent MCMV recombinant viruses were prepared, each of which possesses an M33 open reading frame which has been disrupted with the beta-galactosidase gene. While the recombinant M33 null viruses showed no phenotypic differences in replication from wild-type MCMV in primary mouse embryo fibroblasts *in vitro*, they showed severely restricted growth in the salivary glands of infected mice. These data suggest that M33 plays an important role *in vivo*, in particular in the dissemination to or replication in the salivary gland, and provide the first evidence for the function of a viral GCR homolog *in vivo*.

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08822795 96177128 PMID: 8599195

Isolation and characterization of herpes simplex virus type 1 mutants defective in the UL6 gene.

Patel A H; Rixon F J; Cunningham C; Davison A J
MRC Virology Unit, Institute of Virology, Glasgow, United Kingdom.
Virology (UNITED STATES) Mar 1 1996, 217 (1) p111-23, ISSN 0042-6822 Journal Code: 0110674

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

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Previous studies have shown that the protein encoded by herpes simplex virus type 1 (HSV-1) gene UL6 is required for processing and packaging of replicated viral DNA and is a minor component of virions and capsids. In this report, we describe the construction of UL6- HSV-1 mutants with a disrupted UL6 gene using complementing cells and show that they fail to synthesize the UL6 protein or produce infectious virus in noncomplementing cells. The mutants synthesized but failed to process and encapsidate viral DNA and accumulated only immature capsids which lacked the UL6 protein. Immunofluorescence analysis showed that the UL6 protein, when expressed transiently in transfected cells in the absence of other HSV-1 proteins, is localized exclusively to the nucleus. We also investigated an HSV-1 mutant with a defect in gene UL33, the product of which is also thought to be involved in viral DNA processing and packaging. The phenotype of this mutant on noncomplementing cells with regard to failure to process and encapsidate viral DNA, accumulation of immature capsids, and inability to produce infectious virus was the same as that of UL6- viruses. This mutant, however, produced capsids containing the UL6 protein, indicating that association of the UL6 protein with the capsid is independent of the UL33

protein.

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\$32.09 Estimated cost File357

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\$6.06 TELNET

\$56.13 Estimated cost this search

\$56.50 Estimated total session cost 4.501 DialUnits

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